Survival of Springtails *Hypogastrura tullbergi* and *Proisotoma minuta* on Fungal and Bacterial Diets[1]

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ABSTRACT

Two species of soil-borne Collembola, *Hypogastrura tullbergi* Schäffer and *Proisotoma minuta* Tullberg, which in stock cultures feed on *Cladosporium cladosporioides* (Fres.) De Vries, were exposed to 10 species of soil-borne fungi and 7 species of soil-borne bacteria. Both species survived at least 60 days when exposed to *Aspergillus amstelodami* (Marg. Thom and Church), *A. repens* (Corda) Sacc., *A. tamarii* Kita, and *A. terricola* March.; *A. amstelodami* was the most beneficial diet. They died in less than 60 days when exposed to *Aspergillus alliaceus* Thom and Church, *A. clavatus* Desm., and *A. terreus* Thom. More individuals of *P. minuta* than of *H. tullbergi* were alive on *A. chevalieri* after 60 days. None of the collembolans survived after 30 days on the bacterial species. Both species died after 30 days of exposure to a bacterial species, with many dying earlier; the rate of mortality varied according to the bacterial species. The shortest survival occurred on *Flavobacterium aquatile* (Frankland & Frankland). Although Collembola, bacteria and fungi are found in the same environment, collembolans benefit from their association with certain fungal species, but not from their association with bacteria.

Soil-dwelling Collembola in association with mites and other arthropods contribute directly to the humus fraction, and indirectly by decreasing populations of saprophagous mites, bacteria, and fungi, thereby maintaining a balance among species in the complex of soil organisms (Mills and Alley 1973). Although Collembola are not as efficient as enchytraeid worms in breaking down and mixing soils, they serve an important function in mineral turnover, vegetation succession, and decomposition of organic matter. Along with microorganisms, which the Collembola may disseminate, the insects aid in decomposition of organic substances that they themselves cannot assimilate (Butcher et al. 1971).

Field and laboratory studies on soil flora and fauna have shown that close associations exist among Collembola, fungi, and bacteria (Maynard 1951, Poole 1959, Kevan 1962, Butcher et al. 1971, Mills and Sinha 1971). An understanding of the interactions and relative roles of these organisms in the overall productivity of agricultural soils is a prerequisite toward developing sound management practices for such ecosystems. The purpose of this research was to determine the ability of 2 species of Collembola to survive on 10 species of the fungal genus *Aspergillus* and 7 species of bacteria. The 2 species of Collembola, (Maynard 1951, Mills and Sinha 1971, Sinha unpublished) fungi and bacteria used in this study commonly occur in soils (Gilman 1957, Burges 1967).

Materials and Methods

Two species of Collembola, *Hypogastrura tullbergi* Schäffer (Poduridae) and *Proisotoma minuta* Tullberg (Isotomidae), which are commonly found in agricultural soils on Manitoba farms, were collected and maintained for a year in the laboratory as stock cultures. They were reared on a field fungus, *Cladosporium cladosporioides* (Fres.) De Vries, at 17±2°C and 75±5% RH. The RH was controlled in desiccators containing KOH solutions (Solomon 1951). The springtails were surface sterilized by immersion in 1% sodium hypochlorite solution for 2–3 min and then rinsed in sterile water 4–5 times. The scientific names and authorities for the fungal species used in this paper are from Arx (1972) and those of the bacterial species from Breed et al. (1957).

Experiments with Fungi

Ten species of soil- or seed-borne fungi (Fig. 1 and 2) were obtained from stock cultures maintained at the Agriculture Canada Research Station, Winnipeg.

Each of the fungal species was grown axenically on potato-dextrose agar (PDA) at *p*H 6 in 200×20-mm glass tubes plugged with sterile cotton. The cultures were grown in a well-lighted room at 21±2°C for ca. 2 wk, after which 5 surface-sterilized adults were introduced into each tube and maintained at 17±2°C and 75±5% RH. These conditions are identical to those for the rearing of all stock cultures. The RH was controlled by placing the inoculated tubes into incubators maintained at the required RH and temperature. Surface-sterilized Collembola were placed in tubes containing sterile PDA and served as controls. The tubes were examined for dead insects daily for the first 7 days after inoculation and then weekly for 8 wk. Each test contained 5 replicates and was carried out twice for each species.

Experiments with Bacteria

Seven species of common bacteria (Fig. 3 and 4) found in the soil and in decaying organic matter

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(Breed et al. 1957, Buchannon et al. 1966, Semeniuk 1954) were obtained from stock cultures maintained at the Microbiology Department, University of Manitoba, Winnipeg. The bacteria were grown in trypticase soy broth (Bacto-tryptic Soy Broth®, Difco Labs., Detroit) for ca. 1 wk at 30±1°C. Depending upon the density of growth in each culture vessel, ca. 6 ml of each bacterial culture were placed in sterile test tubes (200×20 mm), and plugged with sterile cotton. The tubes were then centrifuged at 4000 rpm for ca. 15 min.

The supernatant was discarded, leaving ca. 3 g of bacteria at the bottom of the tube. The bacterial pellet was cleansed by resuspending it in sterile distilled water, followed by shaking in a vortex mixer. The tube was then centrifuged at 5000 rpm for 20–25 min. The supernatant was decanted and any excess moisture was removed by slight heating of the test tube sides. The tubes were stored overnight at room temperature (ca. 21°C, 30–40% RH).

To eliminate the possibility of death by the Collembola drowning in the wet bacterial mass a gritty surface was provided for the insects to walk on by adding fine mesh (80 mesh per 2.54 cm) autoclaved, sterile sand to the bacterial pellet. To eliminate excessively dry conditions, the tubes were kept at 100% RH for 24 h.

Five surface-sterilized adults of *H. tullbergi* and *P. minuta* were added to each tube of bacteria. Microscopic observations of the tubes revealed that the excreta of the insects did not contaminate the bacterial diets. Control diets consisted of *Cladosporium cladosporioides* grown on PDA in individual test tubes under conditions similar to those of the fungal experiments. Each test contained 5 replicates and was repeated twice. The tubes were examined daily for 1 wk, then weekly for up to 30 days. The results were recorded and averaged for each species.

Results and Discussion

Survival on Fungal Diets

Both collembolan species can survive longer than 60 days on *C. cladosporioides* grown on PDA. *A. amstelodami* was the most favorable of the 10 fungi and *A. alliaceus, A. clavatus,* and *A. terreus* the least favorable for both species of Collembola (Fig. 1 and 2). Collembola did not survive longer than ca. 1 wk on PDA alone (Fig. 1), only a negligible amount of feeding was observed. The survival of some individual collembolans for at least 4 wk indicates that the fungi had some degree of physical interference of fungal hyphae with the movement of the Collembola.

The survival pattern for the 2 collembolan species were similar on *A. clavatus* and *A. repens,* but the rate for the 2 insect species considered collectively is different on each of the 2 diets (Fig. 1D and

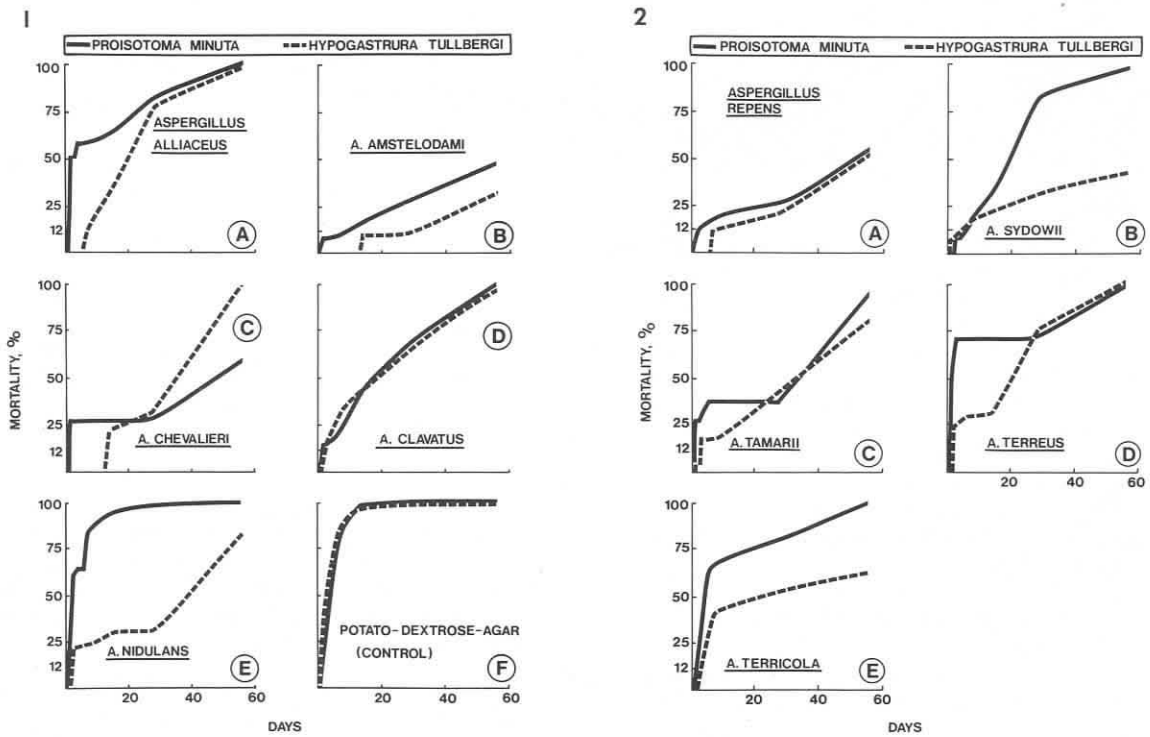


FIG. 1.—Percent mortality of *P. minuta* and *H. tullbergi* on 5 species of fungi and on potato dextrose agar (control).

FIG. 2.—Percent mortality of *P. minuta* and *H. tullbergi* on 6 species of fungi. PDA control is the same as Fig. 1.

2A). The difference in survival rates between species was not conspicuous on *A. nidulans* (Fig. 1 E) and *A. sydowii* (Fig. 2 B).

According to the survival and broad feeding pattern, *H. tullbergi* appears to be the more efficient fungivore of the 2 collembolan species. Maximum survival of *H. tullbergi,* 72% after 8 wk, occurred on *A. amstelodami. H. tullbergi* survived on 6 out of 10 fungi tested, but died on *A. alliaceus, A. chevalieri, A. clavatus,* and *A. terreus.* Mills and Sinha (1971), who tested 5 other species of *Aspergillus,* observed that *H. tullbergi* survived only on *A. versicolor* (Vuill.) Tiraboschi but died on *A. flavus, A. fumigatus* Fresenius, *A. niger* van Tieghem, and *A. ochraceus* Wilhelm. The present study confirms that *H. tullbergi* varies in its reaction to various species of *Aspergillus.* Most species of *Aspergillus* produce mycotoxins with characteristic chemical structure (Scott 1973), which may be related to specific variability in the survival rates of collembolans on *Aspergillus* diets.

P. minuta survived on 5 of the 10 fungi tested. The survival rate of this springtail was uniformly lower than that of *H. tullbergi* on the *A. glaucus* group of fungi (*A. nidulans, A. amstelodami, A. sydowii,* and *A. terricola*) throughout the test period. More individuals of *P. minuta* than of *H. tullbergi* were alive on *A. chevalieri* at the end of the test period. The poorest fungal diet for *P. minuta* was *A. nidulans.*

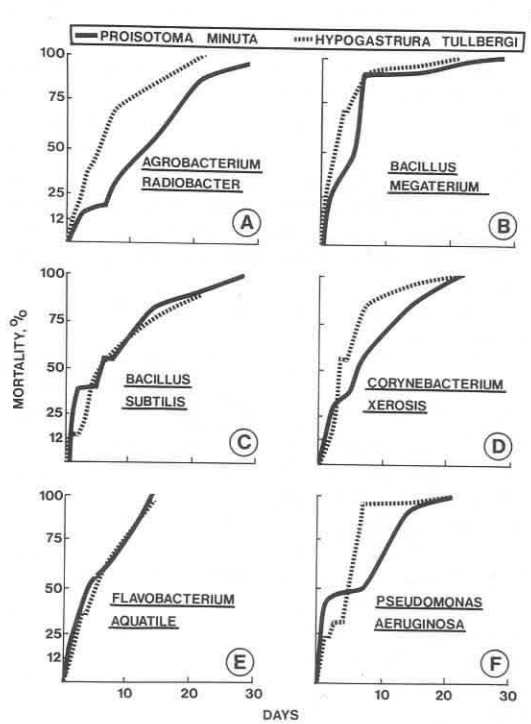
Survival on Bacterial Diets

Over 80% of *P. minuta* and 100% of *H. tullbergi* survived, laid eggs and reproduced in the control (*Cladosporium* on PDA diet) after 30 days (Fig. 4 B). Neither of the collembolan species survived on the bacterial diets for more than 30 days (Fig. 3). Over 50% of the tested individuals of both collembolan species died within the first 2 wk.

Mortality rates for both collembolan species were similar on *Flavobacterium aquatile* (Frankland and Frankland) Bergey et al. (Fig. 3 E), *Bacillus subtilis* (Ehrenberg) Cohn (Fig. 3 C), *Corynebacterium xerosis* (Lehmann and Neumann) (Fig. 3 D), and *Pseudomonas aeruginosa* (Schroeter) Migula (Fig. 3 F). Most individuals of both collembolan species died within 10 days on *Bacillus megatherium* Schroeter; 15 days on *Flavobacterium aquatile;* 22 days on *Corynebacterium xerosis;* and 28 days on *B. subtilis* (Fig. 3 B–F).

H. tullbergi died within 15 days on *Pseudomonas fluorescens* (Trevisan) Migula (Fig. 4 A) and *F. aquatile* (Fig. 3 E), but survived 15 to 30 days on the other bacteria tested. *P. minuta* showed highest mortality on *F. aquatile* (Fig. 3 E) and lowest on *P. fluorescens* (Fig. 4 A).

On all bacterial diets, except *A. radiobacter* (Beijerinck and van Delden) Comm., 50% of *P. minuta* died after 7 days and 100% after 28 days. *P. minuta* and *H. tullbergi* did not benefit appreciably from their association with the bacterial species studied. Collembolan mortality rates on various bacterial species were gradual, except in the case of *F. aquatile,* upon which both collembolan species succumbed quickly. We may conclude from the results of this study that bacterial species found in the soil are toxic to Collembola, and probably have little nutritive value to permit survival.



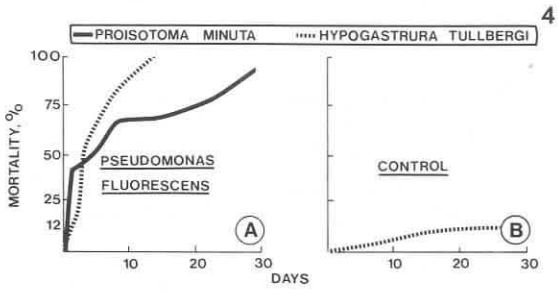


FIG. 3.—Percent mortality of *P. minuta* and *H. tullbergi* on 6 species of bacteria. *C. cladosporioides* control is the same as Fig. 4.

FIG. 4.—Percent mortality of *P. minuta* and *H. tullbergi* on *Pseudomonas fluorescens* and on fungal diet *C. cladosporioides* (control). The zero mortality curve for *P. minuta* is not shown in Fig. 4 B.

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